It is evident from these studies that FF may have different effects on sodium excretion, depending upon degree of potassium depletion. It is also apparent that erroneous conclusions may be drawn from experiments in which urinary excretion of sodium is used as the sole criterion for determining the sodium retaining properties of corticoids. We believe that studies of potassium balance such as those previously reported from our laboratories(2) and of glomerular-tubular functions as reported here are also needed to elucidate the potential long range effects of new adrenal steroids on sodium excretion.

Summary. The loss of potassium attending repeated administration of large doses of nine-alpha-fluorohydrocortisone (FF) to dogs depressed their glomerular filtration rate. In contrast, glomerular function showed a tendency to be augmented in a group of dogs that received supplemental potassium with the steroid. The ability of FF to increase renal tubular reabsorption of sodium was observed under conditions of both potassium depletion and repletion. In the former case sodium retention was enhanced by the reduction in filtration rate but in the latter case no significant retention of sodium occurred, despite its augmented tubular reabsorption.

1. Relman, A. S., Schwartz, W. B., *Am. J. Med.*, 1958, v24, 764.

2. Pentsiaka, J. W., Thomas, B. G. H., Linegar, C. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 712.

3. Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchley, D. W., Lee, R. F., *Am. J. Physiol.*, 1941, v135, 230.

4. Bonsnes, R. W., Taussky, H. H., *J. Biol. Chem.*, 1945, v158, 581.

5. Brownlee, K. A., Industrial Experimentation, 1947, Chemical Publishing Co., Brooklyn, N. Y.

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Multiplication and Cytopathogenicity of Herpes Simplex Virus in Cultures of Feline Renal Cells.* (25298)

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Virus of herpes simplex has been reported to propagate in many tissue culture hosts(1-12); a search of the literature, however, yielded no reference to its growth in feline renal cells. A strain of herpes simplex virus was isolated in our laboratory and its identity confirmed by complement fixation. The purpose of this work was to determine whether the virus would propagate in these cells and to study the cytopathology. This paper describes the cytopathology of herpes simplex virus in cultures of feline renal cells, its behavior in 20 serial passages, and several conditions that influence the type of cell response to this strain of virus.

Materials and methods. Tissue cultures were prepared in 15 × 155 mm tubes and on 11 × 22 mm coverslips in Leighton tubes. Cell cultures of feline renal cells were prepared by the method of Madin(13). Second and third subcultures of human kidney were prepared by trypsin and versene digestion. The nutrient medium was 0.5% lactalbumin in modified Hank's salt solution containing 0.01 M tris (hydroxymethyl aminomethane), adjusted to pH 7.4 and 20% human serum. Prior to inoculation the cultures were rinsed 3 times with Hank's salt solution. The fluid was replaced with 0.5% lactalbumin hydrolysate containing 5% lamb serum. Human amnion cells (FL)(14) were grown in Eagle's medium containing 20% human serum. At the time of inoculation, maintenance medium, consisting of Eagle's basal medium plus 3% horse serum, was placed on the cultures. Streptomycin (0.5 mg/ml) and penicillin (500 u/ml) were incorporated in all media.

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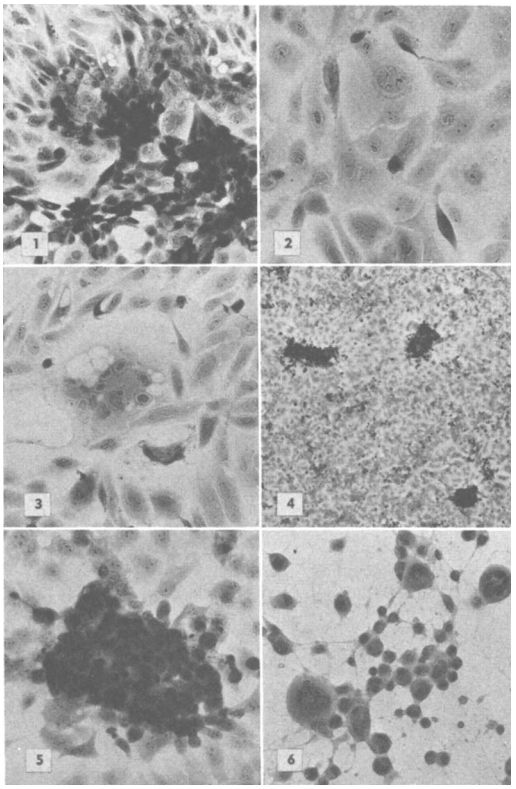

FIG. 1. Proliferative type of lesion in cultures of feline renal cells infected with herpes virus. H & E ×93, AFIP-MIS 58-13112.

FIG. 2. Different stages of nuclear alteration induced by herpes virus in cultures of feline renal cells. H & E ×125, AFIP-MIS 59-2930.

FIG. 3. Multinucleated cell with inclusion bodies in culture of feline renal cells. H & E ×100, AFIP-MIS 59-2931.

FIG. 4. Proliferative type lesion in FL cells infected with herpes virus. H & E ×25, AFIP-MIS 59-2928.

FIG. 5. Same culture as Fig. 4, demonstrating intranuclear inclusion bodies. H & E ×125, AFIP-MIS 59-2927.

FIG. 6. Multinucleated cells in human kidney cell culture infected with herpes. H & E ×100, AFIP-MIS 59-2932.

Titrations of this virus were made in tissue culture by inoculation of 0.1 ml of decimal dilutions into each of 4 tubes. The 50% cytopathogenic end point ($TCID_{50}$) was calculated by the method of Reed and Muench (15). Virus titrations were also made in 21-day-old Swiss albino mice by intracranial inoculation of 0.03 ml of 10-fold dilutions into groups of 6 mice. The mice were observed daily for 3 weeks, and the ID_{50} was calculated by the method of Reed and Muench. The original virus was isolated from a herpetic lesion in primary cultures of human amnion. After one intracerebral passage in mice, the virus possessed an ID_{50} for mice of $< 10^{-1}/0.03$ ml as a 20% brain suspension. The initial inoculum for the feline renal cultures was 0.1 ml of the mouse brain suspension. Subsequent passages were made by transferring 0.1 ml of the undiluted tissue fluid into each of 2 feline renal cell cultures. The fluids from the 2 tubes were pooled and stored at −60°C. Coverslip preparations for studying the cytologic changes were stained with hematoxylin and eosin after fixation in Bouin's fluid.

Results. Cytopathogenic changes (CPC) of the proliferative type (Fig. 1) were first observed in cultures of feline renal cells 6 days following the initial inoculation of the mouse brain suspension. The fluids were pooled and passed on the 8th day. In the 2nd feline kidney passage, cell degeneration was present on the 2nd day and the fluid was passed on the 4th day. In all subsequent passages, CPC was observed on the 1st and 2nd days, and transfers were made on either the 2nd or 3rd day.

Results of virus titrations in both mice and cultures of feline renal cells of 10th, 15th, and 20th tissue culture passages are given in Table I.

With an inoculum of 100 $TCID_{50}$ of virus, lesions were first seen in the unstained preparations at 39 hours. These lesions consisted of small foci of rounded refractile cells, which appeared to pile. These areas increased in size, and between 46 and 63 hours new or secondary foci appeared in culture. At this time giant cells were present, and spaces developed in the cell layer. The degeneration generally becomes more diffuse, and eventually the cells are released from the glass surface.

TABLE I. Comparative Titrations of Herpes Simplex Virus Propagated in Cultures of Feline Renal Cells.

No. of virus passage	Virus titer	
	Mice	Feline renal cells
10	2.5 *	5.75†
15	3.83	5.66
20	3.86	5.23

* Log LD_{50}, Intracer. inoculation of 0.03 ml.
† Log $TCID_{50}$/0.1 ml of inoculum.

In stained preparations small foci involving a few cells exhibiting nuclear alterations are evident as early as 22 hours. The early lesion consists of cells with a slightly granular nucleus in which the nucleoli are small or inapparent. In some cells the nucleus is enlarged and the nuclear space is filled with a homogeneous, light-blue material containing numerous small, dark bodies. As the lesion progresses, these basophilic bodies marginate. The nuclear material condenses, and by 39 hours a spherical to oval inclusion is formed within the shrunken but intact nuclear membrane. The inclusion is surrounded by a clear space or halo. In some cells the nuclear material forms small aggregates interlaced with chromatin, which appears as a fine reticulated network. A number of cells exhibiting various stages of nuclear alteration are illustrated in Fig. 2.

At 22 hours post inoculation a few small multinucleated cells containing 2 or 3 nuclei are present. Although some vacuolation occurs in the cytoplasm at this time, it is not considered to be a specific manifestation of herpes infection. Terminally, the cytoplasm clumps around the nucleus and is granular and eosinophilic. When this occurs, the cells may appear round or elongated, with or without protoplasmic processes.

A typical giant cell is composed of an aggregate (syncytium) of cells that have lost their cytoplasmic identity. In the early stage of syncytium formation, the cytoplasm becomes pale and somewhat vacuolated. Most of the nuclei in these giant cells contain inclusions (Fig. 3). Giant cells are most numerous 86 hours after infection.

This study shows that primary cultures of feline renal cells support the multiplication of herpes simplex virus. The 20th tissue culture passage represents a 1×10^{-20} cumulative dilution of the original virus inoculum. Replicate titrations in cultures of feline renal cells show a $TCID_{50}$ of $10^{-5.23}$ to $10^{-5.75}$/0.1 ml. There was a considerable increase in the LD_{50} of the tissue culture adapted virus when titrated into mice (Table I). Identification of the material from the 10th and 20th virus passages was reaffirmed with known herpes immune serum, utilizing the complement fixation and neutralization tests. In addition the virus maintained its infectivity for the embryonated egg. Characteristic intranuclear inclusion bodies were demonstrated in both the chorioallantoic membrane of the embryonated egg and in the infected mouse brain.

Discussion. Virus of herpes simplex has been reported to propagate and elicit a cytopathogenic change in a variety of tissue culture hosts. A review of the literature reveals that the cytopathic changes resulting from herpes infection are primarily of 3 types. These types, as described by Gray, *et al.*(16), are the (1) proliferative, (2) nonproliferative, and (3) giant cell types. In this study we produced all 3 types of CPC with one isolate of virus.

In the human amnion cultures in which the virus was originally isolated, the cytopathic change was of the nonproliferative type. Distinct foci of the proliferative type were seen in cultures of feline renal cells after inoculation with a large amount of virus, whereas a small quantity of virus produced both the proliferative and giant cell types of CPC in equal proportions. The lesion was of the proliferative type in the FL cultures regardless of amount of inoculum (Fig. 4 and 5). The giant cell type was the predominant cytopathic change in human kidney cultures when a large inoculum was given (Fig. 6).

These results indicate that the type of lesion produced is not dependent entirely on strain of virus used but is also influenced by type of cell culture and amount of the inoculum, as shown above. No attempt to clone the virus was made, and it is possible that a mixture of cytopathogenic types of virus may exist. Although the different cytopathogenic changes noted on the various host cells do not rule out this possibility, this study emphasizes the value of using diverse cell systems.

We rarely observed well-developed intranuclear inclusions in our passages of the strain of herpes in human kidney cells. In this respect our results differ from those of Doane *et al.*(5), who report that typical herpetic inclusions were common in human kidney with their strain of virus.

Macroscopic plaques were produced with

our strain of virus in cultures of feline renal cells in the presence of specific antibody. The presence of antibody did not alter the type of lesion when a large inoculum was used to infect the cultures. The microscopic plaques produced in the FL cells were similar in appearance to those described in HeLa cells by Farnham(17).

Summary. The virus of herpes simplex was passed serially 20 times in cultures of feline renal cells. Multiplication of the virus was demonstrated when infectivity titers were measured in mice and in tissue culture. The adapted virus induced cytopathogenic changes in cell cultures of feline kidney, primary human amnion, human kidney, and FL cells. Several conditions which appear to influence the type of cytopathogenic change in cell cultures are discussed.

1. Randall, L. T., Coates, M. S., *J. Infect. Dis.*, 1942, v71, 83.

2. Scott, T. F. McN., Burgoon, C. F., Coriell, L. L., Blank, H., *J. Immunol.*, 1953, v71, 385.

3. Scherer, W. F., *Am. J. Path.*, 1953, v29, 113.

4. Scherer, W. F., Syverton, J. T., *ibid.*, 1954, v30, 1957.

5. Doane, F., Rhodes, A. J., Ormsby, H. L., *Am. J. Ophthal.*, 1955, v40, 189.

6. Barski, G., Lamy, M., Lepine, P., *Ann. Inst. Pasteur*, 1955, v89, 415.

7. Sosa-Martinez, J., Gutierrez-Villegas, L., Sosa, R. M., *J. Bact.*, 1955, v70, 391.

8. Eagle, H., Habel, K., Rowe, W. P., Huebner, R. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 361.

9. McLaren, L. C., Syverton, J. T., *J. Immunol.*, 1957, v79, 484.

10. Takemoto, K. K., Lerner, A. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 179.

11. Wheeler, C. E., *J. Immunol.*, 1958, v81, 98.

12. Wright, B. S., Sagik, B. P., *Virology*, 1958, v5, 573.

13. Madin, S. H., Andriese, P. C., Darby, N., *Am. J. Vet. Res.*, 1957, v18, 932.

14. Fogh, J., Lund, R. O., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 532.

15. Reed, L. F., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

16. Gray, A., Tokumaru, T., Scott, T. F. McN., *Arch. f. d. Gesamte Virusforsch.*, 1957, v8, 59.

17. Farnham, A. E., *Virology*, 1958, v6, 317.

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Movement of Radiostrontium Through Intestinal Tract of Fed or Fasted Rats.* (25299)

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During a study of radiostrontium absorption in rats, a simple method was developed for determining its progress through the intestinal tract. The method should prove equally of value in studies using other radioactive substances. It is particularly useful for estimating the amount of radioisotope available for absorption in different segments of the tract at various times after oral administration.

Methods. Adult female rats of Wistar strain were given 3 μc of $Sr^{89}Cl_2$ in 0.75 ml water by stomach tube. One group had been fasted during 24 hours prior to experiment, while the other group was fed *ad lib.* Both groups were subsequently given water *ad lib.*, but no food. They were killed in groups of 4 at ½, 1, 2, 4, 6, and 24 hours, and the intestinal tracts removed and laid out in a straight line. The radioactive material present in successive 1 cm lengths of the gut was determined using a collimated Geiger counting tube, a counting ratemeter, and graphic recorder. The gut can be counted continuously by placing it on moving paper of a kymograph with extender, fixed so that the paper is horizontal.

Results. Fig. 1 shows distribution of radiostrontium at different time intervals in representative animals from the fasted group. Fig.

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